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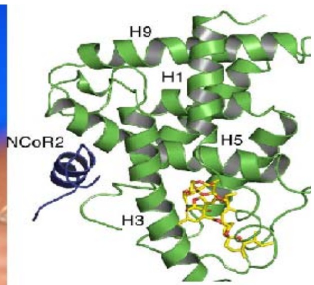

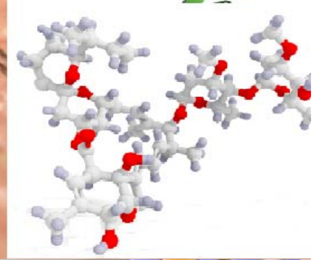
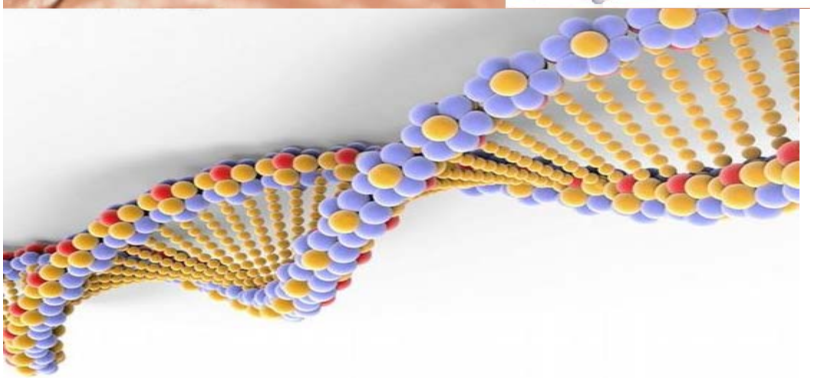
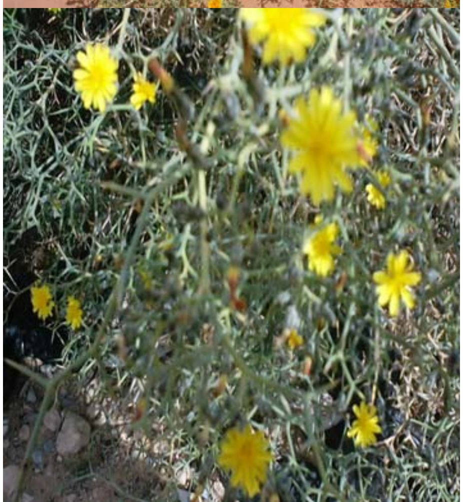
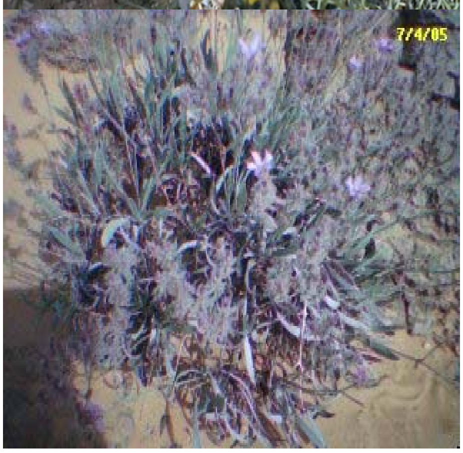

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Seasonal variation of essential oil yield and composition of *Juniperus phoenicea* grown at Djebel Amour region, Algeria

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Abstract. *Yield and composition of the essential oil obtained by hydrodistillation from the aerial parts of juniperus phoenicea (Cupressaceae) have been studied. Plant material has been harvested during three different vegetative stages (before flowering, flowering and after flowering) to look for some correlation between composition and vegetative stage. The yields of oils in different stages were in the order of: before flowering (0.24%), flowering (0.74%) and after-flowering (0.40%). The oils were analyzed by gas chromatography-mass spectrometry. In total,30,35and 35 constituents were identified and quantified in the oil of before flowering, flowering and after flowering plants, representing 99.44, 99.93 and 99.48% of the oils, respectively. a-pinene, a-phellandrene, Linalool, and Caryophyllene oxide were the main compounds in all samples. Monoterpenes were the main group of compounds in before flowering (59.88%), flowering (68.16%) and after flowering (69.39%) stages.*

Key Words: *Juniperus phoenicea, Essential oil, GC/MS, Hydrodistillation*

1. Introduction

Essential oils are complex mixtures, constituted by terpenoid hydrocarbons, oxygenated terpenes and sesquiterpenes. They originate from the plant secondary metabolism and are responsible for their characteristic aroma. The species *Juniperus phoenicea* is considered as an important medicinal plant largely used in traditional medicine. Its leaves are used in the form of decoction to treat diarrhea, rheumatism and diabetes [1,2]. The mixture of leaves and berries of this plant is used as an oral hypoglycaemic agent [3], whereas the leaves are used against bronco-pulmonary disease and as a diuretic[1]. Previously, from the genus *Juniperus* some terpenoids have been isolated [4-7] neolignans

[4] and flavonoids [8].There are many paper reports on chemical compositions of leaves and berries of essential oils of J. *phoenicea* grown in north Mediterranean basin[9]. In Morocco[10,11]; in Egypt[12], in Tunisia [13,14], in Algeria [8,15,16]. All oils of *J. phoenicea* have a high content of α-pinene. Data reported by [17], on the chemical composition of the essential oil from Spanish *Juniperus phoenicea* leaves showed that it was dominated by α-pinene (28.3%) , β-phellandrene (25.3%), myrcene (7.2%),and α-phellandrene (4.1%)[18]. Vidrich and Michelozzi reported 1,8-cineol, α -pinene, and borneol as major components for an oil from an Italian J. *phoenicea* [19]. Rezzi et al. studied the composition of the essential oil from the leaves of *Juniperus phoenicea* from Corsica, and they found two different main compositions, identified ascluster I and cluster II, with the former being rich in α -pinene (70%) and the latter richin α -pinene (33%), β-phellandrene (21.1%) , and α-terpenyl acetate (8.2%) [20]. The essential oil composition of J. *phoenicea* depends on organs, seasons and methods [9,13]. This paper reports the first study of the seasonal variation of the essential oil composition of aerial parts of *juniperus phoenicea* in different growth stages.

2. Materials and methods

2.1. Plant material

Aerial parts of *J. Phoenicea* were collected from Djabel Amour region, Central part of the Algerian saharan Atlas ((at 100 km at the west of Laghouat), during in three different times of growth plant (before flowering, flowering and after flowering stage) respectively, in April, October and January 2013, and were kept in dark at room temperature. The plants were identified with the contribution of the members of the laboratory of Process Engineering, University of Laghouat.

2.2. Isolation of the essential oil

The aerial parts (100 g) were dried at 25°C in the shade and subjected to hydrodistillation using a Clevenger-type apparatus for 4 h. The oil was dried with anhydrous sodium sulfate, weighed and stored at 4- 6°C in dark until use.

2.3. GC Analysis:

The essential oils were analyzed using a Shimadzu GC-2010 Gas chromatography equipped with a flame ionization detector (FID) and a DB-5 capillary column (30m x 0.25m, 1µm film thickness). Nitrogen was used as the carrier gas and the injector and detector temperatures were set at 220 and 280 °C respectively. The oven temperature was programmed from 60 to 230 °C at 3°C min^{-1} and finally held at 230 °C for 10 min. The volume of oil injected was 1 µl. Peak areas and retention times were measured by electronic integration.

2.4. GC/MS Analysis:

GC/MS data was obtained on the Gas Chromatography-Mass Spectrometry (GC-MS)-2010 Plus Shimadzu making use of the same column. The ion source temperature was kept at 250ºC with interface temperature at 280ºC. The carrier gas used was helium. The temperature programming was same as in case of GC. Quantitative results are mean data derived from GC analysis. The mass range was 40 to 850 Dalton.

2.5. Identification of components

The linear retention indices for all the compounds were determined by coinjection of the sample with a solution containing the homologous series of C8 – C22 n-alkanes. The individual constituents were identified by their identical retention indices, referring to known compounds from the literature [17] and also by comparing their mass spectra with either the known compounds or with the Wiley mass spectral database.

3. Results and discussion

The essential oil contents of the aerial parts of *J. phoenicea,* obtained by hydro distillation,were 0.24%, 0.74% and 0.40% in before flowering, flowering and after flowering stages respectively, calculated on a dry weight basis. The components of the essential oils are reported in Table 1. Thirty components accounting for 99.44 % of the total composition were identified in before flowering stage. The major constituents of this oil were α-pinene (29.77 %), manoyl oxide (9.83%), α-phellandrene (7.65%), linalool (5.10%), caryophyllene oxide (5.05%) and elemol (4.15%). In the volatile of flowering stage, thirty-five compounds amounting 99.93 % of the total components were identified which included α - pinene (36.58 %), α-phellandrene (9.40%), caryophyllene oxide (5.54%), elemol (3.96%), β-Caryophyllene (3.72%), Linalool (3.65%) and β-pinene (3.17%) as main components. In the oil obtained from after flowering stage, thirty-five components were identified, which represented about 99.48 % of the total composition. α-pinene (33.29%), α-phellandrene (10.88%), β-myrcene (3.24%), β-pinene and Linalool (3.12%) were the principal components of this oil.

The majority of the identified compounds belonged to the monoterpene fraction (Table 2), with percentages ranging from 59.88% in before flowering stage, to 68.16 % in the flowering stage and 69.39% in after flowering stage. The hydrocarbons fraction was mainly composed of monoterpenes and the flowering stage oil had the highest percentage of hydrocarbons monoterpene. The results from this study show that oils obtained from the different phenological stages have nearly similar compositions; the main compounds were α-pinene, α-phellandrene, Linalool, and Caryophyllene oxide. Thus the time of harvesting of this plant does not have a major effect on the chemical composition of the essential oil, but it affects the essential oil content of the plant. The flowering stage is the best time for harvesting the plant because at this time the plant contains the highest percentage of essential oil.

4. Conclusion

A comparison of the chemical composition of the essential oil from the aerial parts of *J. phoenicea*, at three stages of development shows that there are little differences in composition and major components, α-pinene, α-phellandrene, Linalool, and Caryophyllene oxide were the main compounds in all samples. Thus the time of harvesting of this plant does not have a major effect on chemical composition of the essential oil but it effects on the essential oil content of the plant and the flowering stage is the best time for harvesting the plant and obtaining the essential oil because at this time the plant contains highest percent of the essential oil.

Table 1. The chemical composition of the essential oils of J. *phoenicea* in different growth stages.

No.	Components	KI	Before flowering stage	Flowering stage	After flowering stage
1	Tricyclene	919	-	1.01	0.62
2	α – Pinene	939	29.77	36.58	33.29
3	Camphene	953	0.94	0.99	1.82
4	Verbenene	967	1.04	1.09	0.52
5	β – Pinene	981	2.25	3.17	3.12
6	β – myrcene	992	1.24	1.46	3.24
7	α – phellandrene	1005	7.65	9.40	10.88
8	p-Cymene	1027	1.25	1.30	2.10
9	γ-Terpinene	1056	0.15	0.20	0.49
10	Linalool	1074	5.10	3.65	3.12
11	Fenchone	1087	1.14	-	0.91
12	Trans-Rose oxide	1111	0.49	0.41	0.28
13	Trans-Pinocarveol	1139	1.34	1.47	1.05
14	Camphor	1143	-	0.39	1.59
15	Pinocarvone	1162	0.36	0.23	-
16	Naphthalene	1179	0.26	0.50	0.59
17	Citronellol	1233	3.34	2.94	1.94
18	Linalyl acetate	1261	3.36	1.66	2.16
19	Isopulegyl acetate	1273	-	0.84	1.42

20	γ-Elemene	1336	-	0.43	0.36
21	α−copaene	1376	1.49	1.02	1.05
22	β−bourbonene	1384	t	0.61	0.32
23	β -Elemene	1391	1.44	1.52	0.96
24	β-Caryophyllene	1418	2.80	3.72	3.79
25	α-cubebene	1451	1.27	1.33	1.16
26	α-Humulene	1467	-	1.95	2.02
27	Germacrene D	1480	1.44	1.65	1.88
28	α-amorphene	1514	1.16	1.50	2.04
29	γ−Cadinene	1520	-	0.12	1.53
30	δ−Cadinene	1524	-	-	3.84
31	Valencene	1539	5.05	-	-
32	Elemol	1547	4.15	3.96	-
33	Germacrene B	1560	-	-	2.38
34	Citronellyl acetate	1563	0.70	0.87	0.25
35	Caryophyllene oxide	1581	5.05	5.54	2.61
36	Humulene oxide	1596	2.19	2.41	1.13
37	β-Eudesmol	1630	3.31	3.47	3.08
38	Spathulenol	1619	0.31	0.22	-
39	Manoyl oxide	1989	9.83	2.43	1.91
	Total identified		99.44	99.93	99.48
	Yield (%)		0.24	0.74	0.40

KI = linear Kovats Index on DB-5 column.

Table 2. Percentages of the main chemical classes of volatiles.

Chemical class	Before flowering stage	Flowering stage	After flowering stage
Monoterpene hydrocarbons	50.44	60.11	60.41
Oxygenated monoterpenes	9.44	8.05	8.98
Sesquiterpene hydrocarbons	14.65	13.85	21.33
Oxygenated sesquiterpenes	24.39	18.03	8.73

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